PCT/EP03/13514

BUNDESREPUBLIK DEUTSCHLAND

10/536950

Prioritätsbescheinigung über die Einreichung einer Patentanmeldung

Aktenzeichen:	102 55 861.2
Anmeldetag:	29. November 2002
Anmelder/Inhaber:	Axxima Pharmaceuticals AG, Planegg/DE
Bezeichnung:	Compounds and substances useful against Hepatitis C virus infections
IPC:	A 61 K 33/04

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der ursprünglichen Unterlagen dieser Patentanmeldung.

München, den 19. Dezember 2003
Deutsches Patent- und Markenamt
Der Präsident
Im Auftrag

Nitschke

A 9161
06/00
EDV-L

Compounds and substances useful against Hepatitis C virus infections

Specification

The present invention relates to chemical compounds and substances which are effective against Hepatitis C virus (HCV) infections. In particular, the present invention relates to compositions comprising said compounds and/or substances , to methods for preventing HCV infections as well use of the compounds and/or substances for the preparation of compositions useful for the prophylaxis and/or treatment of HCV infections.

Background of the invention

Hepatitis C Virus (HCV) infection is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. The WHO estimates that approximately 3% of the world population, or 170 million people, have been infected with the Hepatitis C Virus. In the U.S., an estimated 3.9 million Americans have been infected (CDC fact sheet Sept. 2000). Over 80% of HCV-infected individuals develop chronic hepatitis, which is associated with disease states ranging from asymptomatic carrier states to repeated inflammation of the liver and serious chronic liver disease. Over the course of 20 years, more than 20% of chronic HCV-patients are expected to be at risk to develop cirrhosis or progress to hepatocellular carcinoma. Liver failure from chronic hepatitis C is the leading indicator for liver transplantation. Excluding transplantation, the CDC estimates that medical and work-loss cost for HCV annually are around US-$ 600 million. HCV is transmitted primarily by blood and blood products. Due to routine screening of the blood supplies from mid-1992, new transfusion-related cases are exceedingly rare and have been surpassed by injection drug use as the highest risk factor for acquiring the virus. There is also a sexual, however inefficient, route of transmission, and a 6% rate of transmission from infected mothers to their children, which is higher in case of HIV co-infection. In a certain percentage of infections, the mode of transmission remains unknown. In spite of the significant decline in incidence in the 1990's, the number of deaths (estimated deaths annually at the moment: 8000 to 10,000 in U.S.) and severe disease due to HCV is anticipated to triple in the next 10 to 20 years (sources: CDC fact sheet (accessed 12/12/00); Houghton M. Hepatitis C Viruses. In BN Fields, DM Knipe, PM Howley (ed.) Fields Virology. 1996. Lippencott-Raven Pub., Philadelphia; Rosen HR and Gretch DR, Molecular

Medicine Today Vol5, 393, Sept. 99; Science 285, 26, July 99: News Focus: The scientific challenge of Hepatitis C; Wong JB et al, Am J Public Health, 90, 1562, Oct 2000: Estimating future hepatitis C morbidity, mortality, and costs in the United States).

According to the announcement from the EASL (European Association for the Study of the Liver) International Consensus Conference on Hepatitis C (February 26-28, 1999, Paris, France), combination therapy of alpha interferon and ribavirin is the recommended treatment for naive patients. Monotherapy with interferon has also been approved by the FDA, but the sustained response rate (HCV RNA remains undetectable in the serum for more than 6 months after end of therapy) is only 15 to 20%, in contrast to 35 to 45% with combination therapy. Interferons (Intron A, Schering-Plough; Roferon A, Hoffmann-LaRoche; Wellferon, Glaxo Wellcome; Infergen, Amgen) are injected subcutaneously three times a week, ribavirin (Rebetol, Schering-Plough) is an oral drug given twice a day. Recommended treatment duration is 6 to 12 months, depending on HCV genotype. Experimental forms of slow-release pegylated interferons (Pegasys, Hoffmann-LaRoche; PEG-Intron, Schering-Plough) have shown improvements in response rates (42 to 82% in combination with ribavirin) and application (once-weekly injection) in recent clinical studies (Hepatology 32:4, Pt 2 of 2. Oct 2000; NEJM 343, 1673. Dec 2000; NEJM 343, 1666. Dec 2000). Common side effects of interferon therapy include: e.g. fatigue, muscle aches, head aches, nausea, fever, weight loss, irritability, depression, bone marrow suppression, reversible hair loss. The most common side effects of ribavirin are anemia, fatigue and irritability, itching, skin rash, nasal stuffiness, sinusitis, cough. More serious side effects of mono-and combination therapy occur in less than two percent of patients (NIDDK information: Chronic Hepatitis C: Current Disease Management. accessed 09.12.99). Some of the contraindications to interferon are psychosis or severe depression; neutropenia and/or thrombocytopenia; organ transplantation except liver; symptomatic heart disease; decompensated cirrhosis; uncontrolled seizures. Contraindications to ribavirin are end-stage renal failure; anemia; hemoglobinopathies; severe heart disease; pregnancy; no reliable method of contraception (consensus statement EASL). Moreover, treatment of Hepatitis C virus infection with interferon-alpha is effective in only a minority of individuals. This suggests that the virus may use various tricks to be resistant to interferon.

Experimental treatments that are not new forms of interferon are Maxamine (histamine dihydrochloride, Maxim Pharmaceuticals), which will be combined with

Interferon in phase III studies, VX-497 (Vertex Pharmaceuticals), an IMP dehydrogenase inhibitor, as a less toxic ribavirin substitute in phase II, and amantadine (Endo Labs), an approved influenza drug, as the third component in triple therapy (phase II). Inhibitors for HCV enzymes such as protease inhibitors, RNA polymerase inhibitors, helicase inhibitors as well as ribozymes and antisense RNAs are under preclinical development (Boehringer Ingelheim, Ribozyme Pharmaceuticals, Vertex Pharmaceuticals, Schering-Plough, Hoffmann-LaRoche, Immusol, Merck etc.). No vaccine is available for prevention or therapeutic use, but several companies are trying to develop conventional or DNA vaccines or immunostimulatory agents (e.g. Chiron, Merck/Vical, Epimmune, NABI, Innogenetics). In addition, antibodies against HCV virion have been developed and entered into clinical trials recently (Trimera Co., Israel).

In summary, the available treatment for chronic Hepatitis C is expensive, effective only in a certain percentage of patients and adverse side effects are not uncommon.

Description of the invention

Recent research has revealed how cells communicate with each other to coordinate the growth and maintenance of the multitude of tissues within the human body. A key element of this communication network is the transmission of a signal from the exterior of a cell to its nucleus, which results in the activation or suppression of specific genes. This process is called signal transduction.

Signal transduction at the cellular level refers to the movement of signals from outside the cell to inside. The movement of signals can be simple, like that associated with receptor molecules of the acetylcholine class: receptors that constitute channels which, upon ligand interaction, allow signals to be passed in the form of small ion movement, either into or out of the cell. These ion movements result in changes in the electrical potential of the cells that, in turn, propagates the signal along the cell. More complex signal transduction involves the coupling of ligand-receptor interactions to many intracellular events. These events include phosphorylations by tyrosine kinases and/or serine/threonine kinases. Protein phosphorylations change enzyme activities and protein conformations. The eventual outcome is an alteration in cellular activity and changes in the program of genes expressed within the responding cells.

Signal transducting receptors are of three general classes:

1. Receptors that penetrate the plasma membrane and have intrinsic enzymatic activity:

Receptors that have intrinsic enzymatic activities include those that are tyrosine kinases (e.g. PDGF, insulin, EGF and FGF receptors), tyrosine phosphatases (e.g. CD45 [*cluster determinant-45*] protein of T cells and macrophages), guanylate cyclases (e.g. natriuretic peptide receptors) and serine/threonine kinases (e.g. activin and TGF-beta receptors). Receptors with intrinsic tyrosine kinase activity are capable of autophosphorylation as well as phosphorylation of other substrates.

Additionally, several families of receptors lack intrinsic enzyme activity, yet are coupled to intracellular tyrosine kinases by direct protein-protein interactions. This class of receptors includes all of the cytokine receptors (e. g. the interleukin-2 receptor) as well as the CD4 and CD8 cell surface glycoproteins of T cells and the T cell antigen receptor.

2. Receptors that are coupled, inside the cell, to GTP-binding and hydrolyzing proteins (termed G-proteins):

Receptors of the class that interact with G-proteins all have a structure that is characterized by seven transmembrane spanning domains. These receptors are termed *serpentine* receptors. Examples of this class are the adrenergic receptors, odorant receptors, and certain hormone receptors (e.g. glucagon, angiotensin, vasopressin and bradykinin).

3. Receptors that are found intracellularly and upon ligand binding migrate to the nucleus where the ligand-receptor complex directly affects gene transcription:

The steroid/thyroid hormone receptor superfamily (e.g. glucocorticoid, vitamin D, retinoic acid and thyroid hormone receptors) is a class of proteins that reside in the cytoplasm and bind the lipophilic steroid/thyroid hormones. These hormones are capable of freely penetrating the hydrophobic plasma membrane. Upon binding ligand the hormone-receptor complex translocates to the nucleus and bind to specific DNA sequences resulting in altered transcription rates of the associated gene.

When the message reaches the nucleus via one or several of the pathways described above, it initiates the modulation of specific genes, resulting in the production of RNA and finally proteins that carry out a specific biological function. Disturbed activity of signal transduction molecules may lead to the malfunctioning of cells and disease processes. Specifically, interaction of HCV with host cells is necessary for the virus to replicate.

The present invention is based upon the fact that the human cellular protein glutathione peroxidase-gastrointestinal (P18283) is specifically downregulated as a result of HCV replication in HCV infected host cells. The antiviral prophylactic and/or therapeutic approach described herein focuses on specific chemical substances and compounds that can be used to upregulate the human cellular protein glutathione peroxidase-gastrointestinal. These specific chemical substances and compounds are selenium, selenium salts, Vitamin D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN).

In order to develop new pharmaceutically active compounds, a potential target for medical intervention had to be identified. Thus, processes for finding pharmaceutically effective compounds include target identification. Details for finding a suitable target to deal with HCV infections are described in WO 02/084294.

Target identification is basically the identification of a particular biological component, namely a protein and its association with particular disease states or regulatory systems. A protein identified in a search for a pharmaceutically active chemical compound (drug) that can affect a disease or its symptoms is called a target. Said target is involved in the regulation or control of biological systems and its function can be interfered by with a drug.

The word disease is used herein to refer to an acquired condition or genetic condition. A disease can alter the normal biological system of the body, causing an over or under abundance of chemical compounds (chemical imbalance). The regulatory systems for these chemical compounds involve the use by the body of certain proteins to detect imbalances or cause the body to produce neutralizing compounds in an attempt to restore the chemical balance.

The word body is used herein to refer to any biological system, e.g. human, animal, cells, or cell culture.

It is therefore the object of the present invention to provide compounds, compositions and methods which are effective in the prophylaxis and/or treatment of Hepatitis C virus infections, but which do not show the negative side-effects described above or at least not to the extent reported for known products and methods. The object of the present invention is solved by the teaching of the independent claims. Further advantageous features, aspects and details of the invention are evident from the dependent claims, the description, and the examples of the present application.

Detailed description of the invention

It has been shown previously that the human cellular protein glutathione peroxidase-gastrointestinal is specifically downregulated in a body as a result of HCV infection. This human cellular protein glutathione peroxidase-gastrointestinal has been identified as a diagnostic and therapeutic target for dealing with HCV infection.

Glutathione peroxidase:

Four distinct species of glutathione peroxidase have been identified in mammals to date, the classical cellular enzyme, the phospholipid hydroperoxide metabolizing enzyme, the gastroinestinal tract enzyme and the extracellular plasma enzyme. Their primary structures are poorly related. It has been shown that they are encoded by different genes and have different enzymatic properties. The physiological role of the human plasma enzyme remains still unclear due to the low levels of reduced glutathione in human plasma and the low reactivity of this enzyme.

The human cellular protein glutathione peroxidase-gastrointestinal (GI-GPx) is also known as glutathione peroxidase-related protein 2 (GPRP) or glutathione hydrogen peroxide oxidoreductase. It has been assigned to the Accession Number P18283 and the EC Number 1.11.1.9.

The human cellular protein glutathione peroxidase-gastrointestinal (GI-GPx) catalyzes the reduction of various organic hydroperoxides, as well as hydrogen peroxide, with glutathione (GSH) as hydrogen donor (2 GSH + H_2O_2 ⟶ GS—GS

+ 2 H_2O). It has a molecular weight of 84,000 and 4 subunits per mol of enzyme. The enzyme is useful for enzymatic determination of lipid hydroperoxide.

GI-GPx belongs to the family of selenoproteins and plays an important role in the defense mechanisms of mammals, birds and fish against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate. It has been suggested that this enzyme functions in more times as a mechanism of protecting the cellular membrane system against peroxidative damage and that selenium as an essential trace element which may play an important role in this suggested function of the enzyme. It is known that both vitamin E and Se act as antioxidants also in a common mechanism of oxidative stress as an underlying cause of genetic changes.

Selenium functions within mammalian systems primarily in the form of selenoproteins. Selenoproteins contain selenium as selenocysteine and perform a variety of physiological roles. Seventeen selenoproteins have been identified: cellular or classical glutathione peroxidase; plasma (or extracellular) glutathione peroxidase; phospholipid hydroperoxide glutathione peroxidase; gastrointestinal glutathione peroxidase; selenoprotein *P*; types 1, 2, and 3 iodothyronine deiodinase; selenoprotein W; thioredoxin reductase; and selenophosphate synthetase. Of these, cellular and plasma glutathione peroxidase are the functional parameters used for the assessment of selenium status (D. H. Holben, A. M. Smith, *J. Am. Diet. Assoc.* 1999, 99, 836-843).

Beside vitamin E (DL-α-tocopherol), vitamin C (L-ascorbic acid), co-enzyme Q10, zinc, and selenium a lot of further antioxidants such as N-acetyl-L-cycteine, N-acetyl-S-farnesyl-L-cysteine, Bilirubin, caffeic acid, CAPE, catechin, ceruloplasmin, Coelenterazine, copper diisopropylsalicylate, deferoxamine mesylate, R-(-)-deprenyl, DMNQ, DTPA dianhydride, Ebselen, ellagic acid, (-)-epigallocatechin, L-ergothioneine, EUK-8, Ferritin, glutathione, glutathione monoethylester, α-lipoic acid, Luteolin, Manoalide, MCI-186, MnTBAP, MnTMPyP, morin hydrate, NCO-700, NDGA, p-Nitroblue, propyl gallate, Resveratrol, rutin, silymarin, L-stepholidine, taxifolin, tetrandrine, tocopherol acetate, tocotrienol, Trolox®, U-74389G, U-83836E, and uric acid (all available from Calbiochem, San Diego, CA, U.S.A.) which can be applied for preventing and/or treating HCV infections by compensating at least partially the down-regulation of GI-GPx.

Further antioxidants may be selected from the group of carboxylic acids such as citric acid and phenolic compounds such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), propyl gallate, TBHQ (*tert*-butyl hydroquinone), tocopherols, lecithin, gums and resin guiac, THBP (trihydroxybutyrophenone), thiodipropionic acid and dilauryl thiodipropionate, and glycines.

Oxidative damage is mainly caused by free radicals, preferably reactive oxygen intermediates, derived from normal cellular respiration and oxidative burst produced when phagocytic cells destroy bacteria or virus-infected cells. In order to cope with the constant generation of potentially damaging oxygen radicals, eukaryotic organisms have evolved many defense mechanisms. These include the above-mentioned antioxidants which act as free radicals scavengers and which may interact with GI-GPx and/or may activate, stimulate, and/or increase the expression and/or production of GI-GPx. This advantageous effect of the radicals on the amount of GI-GPx generated in the cells competes with the HCV-induced down-regulation of GI-GPx and supports the cells in their fight against the Hepatits C viruses.

HCV infection studies:

The only reliable experimental HCV infection studies have been performed with chimpanzees. So far, there is no simple cell culture infection system available for HCV. Although a number of reports have been published describing *in vitro* propagation attempts of HCV in primary cells and cell lines, questions remain concerning reproducibility, low levels of expression and properly controlled detection methods (reviewed in J. Gen Virol. 81, 1631; Antiviral Chemistry and Chemotherapy 10, 99). Thus, the replicon system described by Bartenschlager and coworkers (Lohmann et al, Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285, 110. 1999) was used for the studies disclosed herein. This replicon system reproduces a crucial part of the HCV replication cycle which is used as a system for simulating HCV infection. Bartenschlager's group produced bicistronic recombinant RNAs, so-called "replicons", which carry the neomycin-phosphotransferase (NPT) gene as well as a version of the HCV genome where the sequences for the structural HCV proteins were deleted. After transfection of the subgenomic HCV RNA molecules into the human hepatoma cell line HuH-7, cells supporting efficient RNA-dependent RNA replication of the HCV replicons were selected based on co-amplification of the NPT gene and resulting resistance to the antibiotic G-418.

Integration of coding information into the cellular genome was an exclusion criteria for functional replicons. Several lines were established from G-418 resistant clones with autonomously replicating HCV RNAs detectable by Northern Blotting. Minus-strand RNA replication intermediates were detected by Northern Blotting or metabolic radio-labeling, and the production of nonstructural HCV proteins was demonstrated by immuno-precipitation after metabolic labeling or Western Blotting.

Possible influences and/or dependencies of HCV's RNA-dependent RNA replication and nonstructural proteins on host cell transcription are accessible to analysis with the Clontech cDNA arrays used in the methods described herein. HuH-pcDNA3 cells are HuH7 cells resistant to G-418 by integration of a NPT gene-carrying plasmid (pcDNA3, Invitrogen) and serve as negative control. Three replicon lines were analyzed for changes in cellular RNA expression patterns compared to the control line:

- HuH-9-13: cell line with persistant replicon I377/NS3-3'/wt, described in Science 1999, 285, 110-113,
- HuH-5-15: cell line with persistant replicon I389/NS3-3'/wt, described in Science 1999, 285, 110-113,
- HuH-11-7: cell line with persistant replicon I377/NS2-3'/wt, described in Science 1999, 285, 110-113.

These HCV replicon cells serve as a system for simulation of HCV infected cell systems, especially for simulating HCV infected mammals, including humans. Interference of HCV with the cellular signaling events is reflected in differential gene expression when compared to cellular signaling in control cells. Results from this signal transduction microarray analysis revealed significant downregulation of GI-GPx. Radioactively labeled complex cDNA-probes from HCV Replicon cells HuH-9-13, HuH-5-15, and HuH-11-7 were hybridized to cDNA-arrays and compared to hybridizations with cDNA-probes from HuH-pcDNA control cells which did not contain HCV Replicons.

Based on the surprising results reported herein, one aspect of the present invention is directed to specific chemical substances and compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections. Specifically, these specific chemical substances and compounds comprise selenium, selenium salts, Vitamine D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl)

retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN).

Furthermore, the present invention discloses a method for treating Hepatitis C virus infection in an individual comprising the step of administering a pharmaceutically effective amount of at least one of the specific chemical compounds and substances referred to above, which upregulate at least partially the activity of GI-GPx or which upregulate at least partially the production of GI-GPx in the cells.

A similar aspect of the present invention is directed to a method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of at least one of the specific chemical compounds and substances referred to above, which upregulate at least partially the activity of GI-GPx or which upregulate at least partially the production of GI-GPx.

Another aspect of the present invention is to provide a method for regulating the production of Hepatitis C virus in an individual or in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of at least one of the specific chemical compounds and substances referred to above, which at least partially upregulate the activity GI-GPx or which at least partially upregulate the production of GI-GPx in the cells.

In addition to the above-mentioned methods the present invention is also directed to a method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in an individual comprising the step of administering a pharmaceutically effective amount of at least one of the specific chemical compounds and substances referred to above, which activates at least partially GI-GPx or which activates or stimulates the production of GI-GPx in the individual.

Another inventive aspect is related to a method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of at least one of the specific chemical compounds and substances referred to above, which activate at least partially the activity of GI-GPx or which activate or stimulate at least partially the production of GI-GPx.

The term "associated diseases" refers to, for instance, opportunistic infections, liver cirrhosis, liver cancer, hepatocellular carcinoma, or any other diseases that can come along with HCV infection.

The function of GI-GPx is to detoxify peroxides in cells and prevent the cells from oxidative damage. Subjecting HCV infected cells to oxidative stress conditions, preferably induced by paraquat or radicals generated from peroxides, leads to a decreased resistance of HCV infected cells in comparison to uninfected cells against toxicity of radicals. Thus, generating artificial oxidative stress conditions allows selective killing of HCV-infected cells.

Examples for useful radical forming compounds (radical initiators) are bipyridyls such as paraquat, 2,2'-bipyridyl and 4,4'-bipyridyl derivatives, bis-6-(2,2'-bipyridyl)-pyrimidines, tris-(2,2'-bipyridyl)-ruthenium, peroxides such as dibenzoylperoxid, diacetylperoxide, hydrogen peroxide, di-tert.-butylperoxide, or diaza compounds such as diazaisobutyronitril.

Yet another aspect of the present invention is directed to a novel therapeutic composition useful for the prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus and/or associated diseases comprising at least one of the specific chemical substances and compounds selected from the group consisting of selenium, selenium salts, Vitamin D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN). A preferred selenium salt is sodium selenite. Moreover, according to a further preferred aspect of the present invention, the composition my contain a certain amount of all trans retinoic acid.

Further embodiments of the present invention are represented by methods for regulating the production of Hepatitis C virus in an individual or in cells or cell cultures comprising the step of administering an individual or the cells a pharmaceutically effective amount of at least one of the specific chemical substances and compounds selected from the group consisting of selenium, selenium salts, Vitamin D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN), wherein said substance or compound

activates or increases at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially activates or stimulates the production of said human cellular protein glutathione peroxidase-gastrointestinal.

Another aspect of the present invention is directed to novel therapeutic compositions useful within said methods for prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus and/or associated diseases. Said compositions comprise at least one of the specific chemical substances and compounds selected from the group consisting of selenium, selenium salts, Vitamin D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN), capable of increasing the activity of GI-GPx or of activating or stimulating the production and/or expression of GI-GPx.

Said pharmaceutical compositions may further comprise pharmaceutically acceptable carriers, excipients, and/or diluents.

As used herein, the term "activator" refers to any chemical compound capable of upregulating, activating, stimulating, or increasing the amount and/or activity of GI-GPx or its expression.

The term "agent" is used herein as synonym for regulator, inhibitor, and/or activator. Thus, the term "agent" refers to any chemical or biological compound capable of down- or upregulating, de- or increasing, suppressing or stimulating, inactivating or activating, or otherwise regulating or effecting the amount and/or activity of GI-GPx and/or the expression of GI-GPx.

In addition to the role in transmitting genetic information from DNA to proteins, RNA molecules participate actively in many cell processes. Examples are found in translation (rRNA, tRNA, tmRNA), intracellular protein targeting (SRP), nuclear splicing of pre-mRNA (snRNPs), mRNA editing (gRNA), and X-chromosome inactivation (Xist RNA). Each of these RNA molecules acts as a functional product in its own right, without coding any protein. Because RNA molecules can fold into unique shapes with distinct structural features, some RNAs bind to specific proteins or small molecules (as in the ATP-binding aptamer), while others catalyze particular chemical reactions. Thus, RNA aptamers can be used to interact with

GI-GPx and thereby modulate, regulate, activate, or inhibit the activity and biological function of said peroxidase.

As used herein, the term "regulating expression and/or activity" generally refers to any process that functions to control or modulate the quantity or activity (functionality) of a cellular component. Static regulation maintains expression and/or activity at some given level. Upregulation refers to a relative increase in expression and/or activity. Accordingly, downregulation refers to a relative decrease in expression and/or activity. Downregulation is synonymous with inhibition of a given cellular component's activity.

Further aspects of the present invention relate to methods either for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in an individual or in cells or cell cultures comprising the step of administering either the individual or the cells or cell cultures a pharmaceutically effective amount of an agent wherein said agent inhibits or decreases at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

Therapeutics, pharmaceutically active agents or inhibitors, respectively, may be administered to cells from an individual *in vitro*, or may involve *in vivo* administration to the individual. The term "individual" preferably refers to mammals and most preferably to humans. Routes of administration of pharmaceutical preparations to an individual may include oral and parenteral, including dermal, intradermal, intragastral, intracutan, intravasal, intravenous, intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan, rectal, subcutaneous, sublingual, topical or transdermal application, but are not limited the these ways of administration. For instance, the preferred preparations are in administratable form which is suitable for oral application. These administratable forms, for example, include pills, tablets, film tablets, coated tablets, capsules, powders and deposits. Administration to an individual may be in a single dose or in repeated administrations, and may be in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier, binder, lubricant, excipient, diluent and/or adjuvant. Pharmaceutically acceptable salt forms and standard pharmaceutical formulation techniques are well known to persons skilled in the art.

As used herein, a "pharmaceutical effective amount" of a GI-GPx activator is an amount effective to achieve the desired physiological result, either in cells or cell cultures treated *in vitro* or in a subject treated *in vivo*. Specifically, a pharmaceutically effective amount is an amount sufficient to inhibit, for some period of time, one or more of the clinically defined pathological processes associated with the viral infection. The effective amount may vary depending on the specific GI-GPx inhibitor or activator selected, and is also dependent on a variety of factors and conditions related to the subject to be treated and the severity of the infection. For example, if the activator is to be administered *in vivo*, factors such as the age, weight and health of the patient as well as dose response curves and toxicity data obtained in pre-clinical animal work would be among those considered. If the activator is to be contacted with the cells or cell cultures *in vitro*, one would also design a variety of pre-clinical *in vitro* studies to assess such parameters as uptake, half-life, dose, toxicity, etc. The determination of a pharmaceutically effective amount for a given agent is well within the ability of those skilled in the art.

The present disclosure teaches for the first time the upregulation of GI-GPx specifically involved in the viral infection of Hepatitis C virus using specific chemical compounds and substances selected from the group consisting of selenium, selenium salts, Vitamin D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN).

The polypeptide product of gene expression may be assayed to determine the amount of expression as well. Methods for assaying for a protein include, but are not limited to, Western Blotting, immuno-precipitation, radioimmuno assay, immuno-histochemistry and peptide immobilization in an ordered array. It is understood, however, that any method for specifically and quantitatively measuring a specific protein or mRNA product can be used.

The present invention further incorporates by reference in their entirety techniques well known in the field of microarray construction and analysis. These techniques include, but are not limited to, techniques described in the following patents and patent applications describing array of biopolymeric compounds and methods for their fabrication:

U.S. Pat. Nos. 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,436,327; 5,445,934; 5,472,672; 5,527,681; 5,529,756; 5,545,531; 5,554,501; 5,556,752; 5,561,071; 5,559,895; 5,624,711; 5,639,603; 5,658,734; 5,807,522; 6,087,102; WO 93/17126; WO 95/11995; WO 95/35505; EP 742 287; and EP 799 897.

Techniques also include, but are not limited to, techniques described in the following patents and patent application describing methods of using arrays in various applications:

U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049; 5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839; 5,580,732; 5,661,028; 5,994,076; 6,033,860; 6,040,138; 6,040,140; WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317; EP 373 203; and EP 785 280

A robust cell culture system for the hepatitis C virus (HCV) has not been established. For this reason, it is extremely difficult to study how HCV infects cells and to test anti-viral drugs in a model system (the only animals that can be infected are humans and chimpanzees). A major step in devising a culture system for HCV was established by the replicon cell lines (Lohmann, V., Korner, F., Koch, J.-O., Herian, U., Theilmann, L., and Bartenschlager, R. 1999. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science.* 285: 110 - 113). Replication of subgenomic HCV RNAs in cultured hepatocytes were obtained for the first time. These subgenomic replicons are composed of only the part of the HCV genome that encodes the non-structural proteins but are competent to be replicated in cells and synthesize viral proteins. The replicons described in the scientific article of Lohmann et al. cited above and used for the present investigation allows studies of HCV replication, pathogenesis and evolution in cell culture. They may also allow for cell-based testing of certain types of anti-viral drugs.

Recently, gastrointestinal-glutathione peroxidase (GI-GPx) could be validated as target in HCV-replication (see WO 02/084294). As mentioned above, GI-GPx belongs to the family of selenoproteins and plays an important role in the defense mechanisms of eucaryotic cells against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate. It has been suggested that this enzyme functions as a mechanism of

protecting the cellular membrane system against peroxidative damage. Selenium as a necessary trace element suggests the essential function of this enzyme.

Selenium functions within mammalian systems primarily in the form of selenoproteins. Selenoproteins contain selenium as selenocysteine and perform a variety of physiological roles. Seventeen selenoproteins have been identified: cellular or classical glutathione peroxidase; plasma (or extracellular) glutathione peroxidase; phospholipid hydroperoxide glutathione peroxidase; gastrointestinal glutathione peroxidase; selenoprotein *P*; types 1, 2, and 3 iodothyronine deiodinase; selenoprotein W; thioredoxin reductase; and selenophosphate synthetase. Of these, cellular and plasma glutathione peroxidase are the functional parameters used for the assessment of selenium status (D. H. Holben, A. M. Smith, *J. Am. Diet. Assoc.* 1999, 99, 836 - 843).

GI-GPx is drastically down-regulated in HCV replicon cells compared with mock-transfected HuH7 cells. Forcing replicon cells to re-express GI-GPx (e.g. by infection with GI-GPx containing Adenovirus) results in reduction of subgenomic HCV RNA and of the HCV protein NS5a to hardly detectable levels (see WO 02/084294). According to the present invention the knowledge of this inverse correlation was used to develop a method to up-regulate the expression of the cellular, endogenous GI-GPx gene. This up-regulation in replicon cells causes a depletion of HCV.

It is readily apparent to those skilled in the art that other suitable modifications and adaptations of the compositions and methods of the invention described herein are evident and may be made without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

Examples

Reference is made to the Examples of WO 02/084294, which are incorporated herein by reference.

Moreover, as model system for HCV replication there were utilized three replicon cell lines provided by Prof. R. Bartenschlager (University of Heidelberg, FRG). Cultures were treated for various periods of time with all *trans* retinoic acid (RA) for comparative purposes and the other agents selenium, selenium salts, Vitamin D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN) (obtained from Sigma). Levels of expression of GI-GPx was measured on protein level by Western Blotting using antibodies provided by Prof. Brigelius-Flohe (University of Potsdam, FRG) and on RNA level by Northern blotting using GI-GPx-specific oligonucleotides as probes. Levels of HCV RNA were investigated by Northern Blotting using a DNA oligonucleotide complementary to the neomycin phosphotansferase gene as probe. Concentration of the viral protein NS5a was determined by Western Blotting with an NS5a-specific antibody (Biogenesis, UK).

Treatment of replicon cells for three days with all trans retinoic acid (1 µM) had hardly an effect on GI-GPx and HCV expression. However, after seven days of incubation, a drastic up-regulation of GI-GPx on RNA- and protein level (three- to ten-fold) was observed. Concomitantly expression of subgenomic HCV RNA and of viral protein NS5a was downregulated two- to five-fold, depending on the cell line investigated. Furthermore, surprisingly it was found that a further downregulation of HCV-RNA and -NS5a protein was dependent on the addition of selenium or a selenium salt, e.g. sodium selenite (50 nM). This fact implies, that downregulation of HCV was promoted firstly by activation of the GI-GPx gene on transcriptional level by retinoic acid and secondly by the synthesis of selenoprotein(s) for which sodium selenite was needed. Indeed it could be shown that all trans retinoic acid-induced downregulation of HCV is independent of the innate immune response induced by interferon. Thus, all trans retinoic acid did not induce the transcription of PKR (double strand RNA-dependent protein kinase). Severe cytotoxic effects were neither observed for all trans retinoic acid nor for sodium selenite, or both in combination.

The presented findings show that retinoids (in combination with selenium or selenium salts like sodium selenite) can be used for the treatment of HCV-positive patients. Especially the usage of retinoids with high specificity for induction of the GI-GPx, like N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN), are preferred. 4-

HPR and AHPN display significant potential as therapeutic agents in the prophylaxis and treatment of a number of premalignant and malignant conditions in the context of HCV infections. Indeed, the obtained data show that next to all trans retinoic acid other nuclear receptor ligands, like 9-*cis* retinoic acid 9-cis retinoic acid C_1 to C_{10} alkyl esters, 9-cis retinoic acid C_1 to C_{10} alkyl amides, and Vitamin D_3, are also capable of reducing HCV load.

All-*trans* retinoic acid on replicon cells for six days led to an upregulation of GI-GPx RNA and protein due to the fact that the GI-GPx -promoter contains three retinoic acid receptor recognition elements. In the presence of selenium or a selenium salt like sodium selenite a two- to five-fold reduction of HCV-RNA and HCV-NS5a protein was observed in the absence of toxic effects. Moreover, also the specific retinoids, like N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437; AHPN), 9-*cis* retinoic acid, 9-cis retinoic acid C_1 to C_{10} alkyl esters, 9-cis retinoic acid C_1 to C_{10} alkyl amides, and Vitamin D_3 alone or in combination with each other or with selenium or a selenium salt showed a similar effect.

Finally, first preliminary result have shown that 9-cis retinoic acid and its above-mentioned alkyl and amide derivates downregulate HCV RNA significantly better than all trans retinoic acid alone.

Claims

1. Composition useful for the prophylaxis and/or treatment of an individual afflicted with a Hepatitis C virus (HCV) infection and/or at least one disease associated with a HCV infection, said composition comprising at least one agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN).

2. Composition according to claim 1, wherein the selenium salt is sodium selenite.

3. Composition according to claim 1 or 2, wherein the composition further comprises all trans retinoic acid.

4. Composition according to one of claims 1 to 3, further comprising at least one pharmaceutically acceptable carrier, excipient and/or diluent.

5. Use of at least one of the agents selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) for the preparation of a pharmaceutical composition for the treatment and/or prophylaxis of a Hepatitis C virus infection and/or a disease associated with HCV infection.

6. Use according to claim 5, wherein the selenium salt is sodium selenite.

7. Use according to claim 5 or 6, wherein the composition further comprises all trans retinoic acid.

8. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in an individual comprising the step of administering a pharmaceutically effective amount of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic

acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) to the individual.

9. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) to the individual.

10. Method for regulating the production of Hepatitis C virus in an individual comprising the step of administering an individual a pharmaceutically effective amount of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) to the individual.

11. Method for regulating the production of Hepatitis C virus in cells or cell culture comprising the step of administering a pharmaceutically effective amount of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) to the cells or cell culture.

12. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in an individual comprising the step of administering a pharmaceutically effective amount of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) which activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or which activates or stimulates at least partially the production of said human cellular protein glutathione peroxidase-gastrointestinal.

13. Method for preventing and/or treating Hepatitis C virus infection and/or diseases associated with HCV infection in cells or cell cultures comprising the step of administering a pharmaceutically effective amount of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) which activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or which activates or stimulates at least partially the production of said human cellular protein glutathione peroxidase-gastrointestinal.

14. Method for regulating the production of Hepatitis C virus in an individual comprising the step of administering an individual a pharmaceutically effective amount of an agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN), wherein said agent activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially activates or stimulates the production of said human cellular protein glutathione peroxidase-gastrointestinal.

15. Method for regulating the production of Hepatitis C virus in cells or cell culture comprising the step of administering a pharmaceutically effective amount of an agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN), wherein said agent activates at least partially the activity of said human cellular protein glutathione peroxidase-gastrointestinal or wherein said agent at least partially activates or stimulates the production of said human cellular protein glutathione peroxidase-gastrointestinal in the cells or cell culture.

16. Method for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in an individual comprising the step of administering the individual a pharmaceutically effective amount of an agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl

amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN), wherein said agent inhibits at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

17. Method for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in an individual comprising the step of administering the individual a pharmaceutically effective amount of an agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, C_1 - C_{10} alkyl amides of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN), wherein said agent activates at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

18. Method for regulating the expression of the human cellular protein glutathione peroxidase-gastrointestinal in cells or cell culture comprising the step of administering the cells or cell culture a pharmaceutically effective amount of an agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl amide of 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN), wherein said agent activates at least partially the transcription of DNA and/or the translation of RNA encoding said human cellular protein glutathione peroxidase-gastrointestinal.

19. Method for regulating the activity of the human cellular protein glutathione peroxidase-gastrointestinal in an individual comprising the step of administering the individual a pharmaceutically effective amount of an agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl amide of 9-cis retinoic acid, C_1 - C_{10} alkyl amide of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN), wherein said agent interacts with said human cellular protein glutathione peroxidase-gastrointestinal.

20. Method for regulating the activity of the human cellular protein glutathione peroxidase-gastrointestinal in cells or cell culture comprising the step of administering the cells or cell culture a pharmaceutically effective amount of an agent selected from the group consisting of selenium, selenium salts, Vitamin D_3, 9-cis retinoic acid, C_1 - C_{10} alkyl amide of 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN), wherein said agent interacts with said human cellular protein glutathione peroxidase-gastrointestinal.

Abstract

The present invention relates generally to chemical compounds and substances which are effective against Hepatitis C virus (HCV) infections. Moreover, the present invention relates to compositions comprising said compounds and/or substances , to methods for preventing HCV infections as well use of the compounds and/or substances for the preparation of compositions useful for the prophylaxis and/or treatment of HCV infections. Useful compounds and substances according to the invention are selenium, selenium salts, Vitamin D_3 and retinoids, like 9-cis retinoic acid, C_1 - C_{10} alkyl amide of 9-cis retinoic acid, C_1 - C_{10} alkyl esters of 9-cis retinoic acid, N-(4-hydroxyphenyl) retinamide (4-HPR) and 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN).